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Original communication

A method of calculating human deciduous crown formation times and of estimating the chronological ages of stressful events occurring during deciduous enamel formation

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ABSTRACT

Knowledge of deciduous crown formation times is useful in forensic anthropology and when aging juvenile remains from an archaeological context. Until now, histological techniques for calculating enamel formation times in deciduous teeth have been completely dependent on being able to visualise clear daily incremental markings. In the first part of this study we took twenty deciduous teeth where daily incremental markings were easily visible on both aspects of the crown and used these as the basis for generating regression equations to predict enamel formation times. We were then able to use these regression equations to calculate deciduous crown formation times in a further fifty deciduous teeth where it was not possible to see daily increments. We present here new data for deciduous crown formation times based on these regression equations. In the second part of this study these regression formulae were applied blind to teeth from two individuals with known medical histories. The formulae were able to successfully determine the times of prenatal and postnatal enamel formation relative to the neonatal line and also to correctly estimate the ages at which accentuated 'stress lines' occurred during the period of deciduous crown formation.

1. Introduction

Forensic investigations of the remains of infants and young children often include making estimates of an age at death based on dental development. Age estimation of an individual involves first establishing a biological age and then attempting to correlate it with a chronological age. Biological age may be expressed as either 'skeletal age' or 'dental age' and it is generally recognised that '*the relationship between chronological age and dental age is stronger than that for chronological age and skeletal age*,'.[1:3] The dental age of an individual can be estimated by examining the extent of dental eruption or the state of formation or maturation of the developing tooth germ. Of these two, '*formation of teeth appears to be more robust to environmental influences*'.[2:143] However, in order to estimate the dental age of an individual or an isolated tooth, the specimen in question must be compared to a 'known standard'. Unfortunately by doing this certain incompatibilities are inevitably introduced. For this reason, even though the establishment of age at death of juvenile remains can be considered more accurate than establishing the age of death of adult remains,[1,2] due to the short span of time being considered, the aging of juvenile remains based on dental development is nevertheless always only an '*estimation*'.[1:3] Such age estimations are usually derived from direct comparisons between the stage of dental formation of the deciduous teeth of the individual in question, with a similar stage of dental formation in a child of a known age. Although this is the method most commonly used, age estimations have also been derived from histological methods using counts of the daily incremental markings within enamel.[3–5] Although these histological techniques for estimating an age at death are very labour intensive, potentially they can provide more accurate age estimates.[6]

Previous studies that have attempted to establish a chronology for deciduous crown formation times have struggled to define the prenatal age of initial deciduous tooth mineralisation, the timing of birth during enamel formation and the cessation of enamel formation in the same individuals or specimens. Furthermore, different techniques and methods have been used by different authors using material from a variety of sources to try to identify the timing of these events. Unfortunately this has lead to a degree of confusion in the literature. The identification of initial mineralisation depends on the technique used to observe it.[7–10]

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Inconsistencies and inaccuracies incurred in the determination of the actual age of the fetal specimens examined[11,12] and the inclusion of pathological specimens in the sample studied[13–16] have also contributed to the very variable picture of deciduous pre- and postnatal crown formation times. Moreover, in the majority of cases, sample sizes have inevitably been very small, sometimes consisting of only a few individuals,[13–15,17] however a small number of notable more recent studies have used considerably larger sample sizes.[8,12,18]

The main aim of this current study was to develop a simple histological technique which could be used to estimate deciduous crown formation times, without requiring fetal material. We developed a set of regression equations that describe the time taken to form any given thickness of deciduous enamel during crown formation and which can be measured on any longitudinal ground section. These data can then be referred to as 'known standards' and used to help estimate the age of human juvenile remains or isolated deciduous teeth. This work aims to define more clearly the start and finish of enamel matrix secretion in the deciduous crown, in order to improve methods for estimating the age at death of juvenile human remains from forensic, archaeological and even palaeontological contexts.[19] In order to test the usefulness of the equations generated in this study we then blind tested the regression equations on teeth from two individuals with a known medical history of stress events, many of which could be observed as accentuated markings in the enamel. The data presented here are not intended to be taken so much as new improved standards, but rather the methods proposed here offer an example and a solution of how objective data can now be collected from isolated deciduous teeth.

2. Materials and methods

From a sample of approximately 100 longitudinal ground sections of deciduous mandibular teeth sectioned in the true buccolingual axial plane, four of each tooth type were selected that exhibited clearly visible daily cross-striations along the prism paths, between the enamel-dentine junction (EDJ) and the enamel surface ($n = 20$). These sections were made from extracted deciduous teeth collected from dental clinics in the UK and are of multiethnic origins. Any teeth exhibiting pathology or excessive attrition or abrasion were rejected from the sample.

Each aspect (lingual and labial/buccal) of each crown section was then divided into occlusal, lateral and cervical regions (see Fig. 1a). Photomontages were constructed of each of these regions for both aspects. These montages were constructed from a series of overlapping photographic prints taken with an Olympus OM-2N camera loaded with Kodak Gold 200 film attached to a Carl Zeiss Jenamed 2 light microscope with an apochromat 25×/0.65 ∞/0.17-A objective lens. The resulting fieldwidth of a 5 × 7 inch print was 410 μm which is large enough to keep measurement error to a minimum.

Distances of 100 μm, measured along one prism path in each region on each aspect of every crown were indicated on the photomontages (see long broken black line Fig. 1b). A 50 μm zone was included near the surface if the enamel stopped significantly short of a 100 μm measurement.[20] Cumulative daily cross-striation counts were made along each prism path and recorded at every 100 μm or final 50 μm of enamel thickness, from the EDJ to the enamel surface in the occlusal, lateral and cervical regions of each tooth type. This procedure was repeated on both lingual and labial/buccal aspects of each ground section (total prism paths counted $n = 120$).

Linear regression equations were generated from the cumulative cross-striation counts plotted against linear enamel thickness

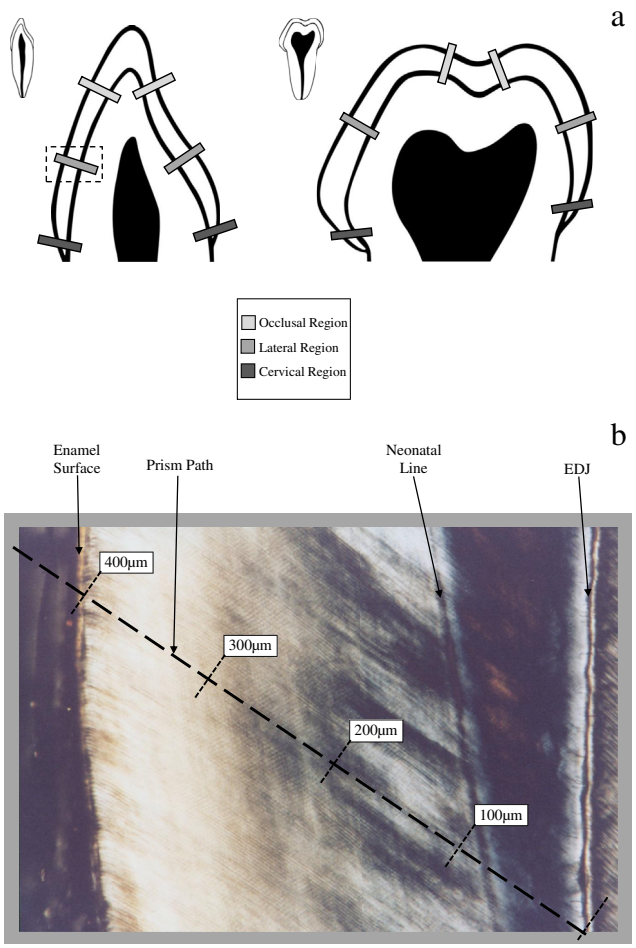

Fig. 1. a. Cervical, lateral and occlusal regions for anterior and posterior teeth as defined in this study. b. Polarised light micrograph of enamel in the lateral region of the labial aspect of a deciduous canine. The long broken black line runs along the prism path from the EDJ to the enamel surface passing through the neonatal line. Counts of enamel cross-striations were made along this prism path at 100 μm intervals (short broken lines) and cumulated.

along a prism path, for each aspect and each region of each tooth type using *Statview* (*Abacus System*™) these were then compared. Independent *t*-tests were performed to determine whether the labial/buccal and lingual aspect of each tooth differed significantly in their number of cross-striations. Three paired *t*-tests were also performed to determine whether there was any significant difference between the three different regions (cervical, lateral and occlusal) on each enamel aspect for each tooth type. Regression equations that combined data from statistically similar regions were then generated.

These combined regression equations were used to produce a reference table of increasing enamel thicknesses for each tooth type with predictions of the average number of days required to produce each thickness from the EDJ to the enamel surface, together with upper and lower 95% confidence limits.

The combined regression equations were next applied to a second sample of ground sections. This second sample consisted of 50 different deciduous ground sections (ten of each tooth type) and was selected from the original collection of 100 ground sections. This sample consisted of sections where the neonatal line and other accentuated striae were visible but where daily incremental markings were not necessarily well preserved.

Photomontages were constructed of the entire enamel crown of each tooth from the second sample. These montages were

constructed from a series of overlapping photographic prints and also taken with an Olympus OM-2N camera loaded with Kodak Gold 200 film attached to a Carl Zeiss Jenamed 2 light microscope with an planachromat Pol 2.5/0.05 ∞/-A objective lens. The resulting fieldwidth of a 5 × 7 inch print was 4060 μm. Following the method of Boyde,[3] Risnes[21] and Dean,[22] prism paths were traced outwards from the EDJ until the occurrence of an accentuated line, this prism length was then measured. The accentuated line was followed back to the EDJ at a lower point on the crown and the procedure was repeated until the end of enamel formation at the cervix of the crown (see Fig. 2).

Measurements were obtained for each prism length from the EDJ to each accentuated line in a sequential manner through the entire enamel crown from cusp to cervix. Each length was then substituted into the appropriate regression formula, which provided the average number of days taken to form each of the enamel prism lengths. The total enamel formation time (crown formation time) is equal to the sum of all of the estimates of enamel formation time along each prism path. The average number of days of enamel formation was calculated for each tooth type together with upper and lower 95% confidence limits.

3. Results

Table 1a shows the independent *t*-tests that were performed to determine whether the labial/buccal and lingual aspect of each tooth differed significantly in the number of cross-striations present. Any value less than 0.0167 indicates a highly significant difference in the number of cross-striations between the two aspects. The independent *t*-tests confirmed that only the first molar showed any statistically significant differences with the buccal enamel appearing to develop at a slower rate than the lingual enamel. This difference appears in both the lateral and the occlusal regions. The other four tooth types did not express any significant differences between the labial/buccal and lingual aspects.

Based on the above results, both aspects of the crown of each tooth type were then treated as being identical and these data were merged together. This merging of the data both increases the sample size and creates a statistically sound data set for the subsequent analyses. In the case of the first molar, the data from the lateral and occlusal regions on the lingual aspect were eliminated from the subsequent analyses. Data from the buccal aspect were retained as the enamel on this aspect is thicker than that on the lingual aspect and it contains the greatest record of time from initiation at the dentine horn until the end of enamel formation at the cervix. Therefore, in a forensic context, the buccal aspect is of more use when trying to establish an estimated age at death, as it potentially offers a longer time line than the lingual enamel.

Unfortunately as the sample size was too small (i.e. less than 5) in the cervical regions of the central incisors and the second molars, an accurate test of significance could not be determined. However as the lateral and occlusal areas of both of these teeth were not significantly different it seems reasonable to suggest that the whole tooth behaves in the same manner and that there was no significant difference between the labial/buccal and lingual aspects in these teeth.

Table 1b shows the paired sample *t*-tests calculated to identify any significant differences between the three regions on the same dental aspect for each tooth type. Any value less than 0.0167 indicates a highly significant difference in the number of cross-

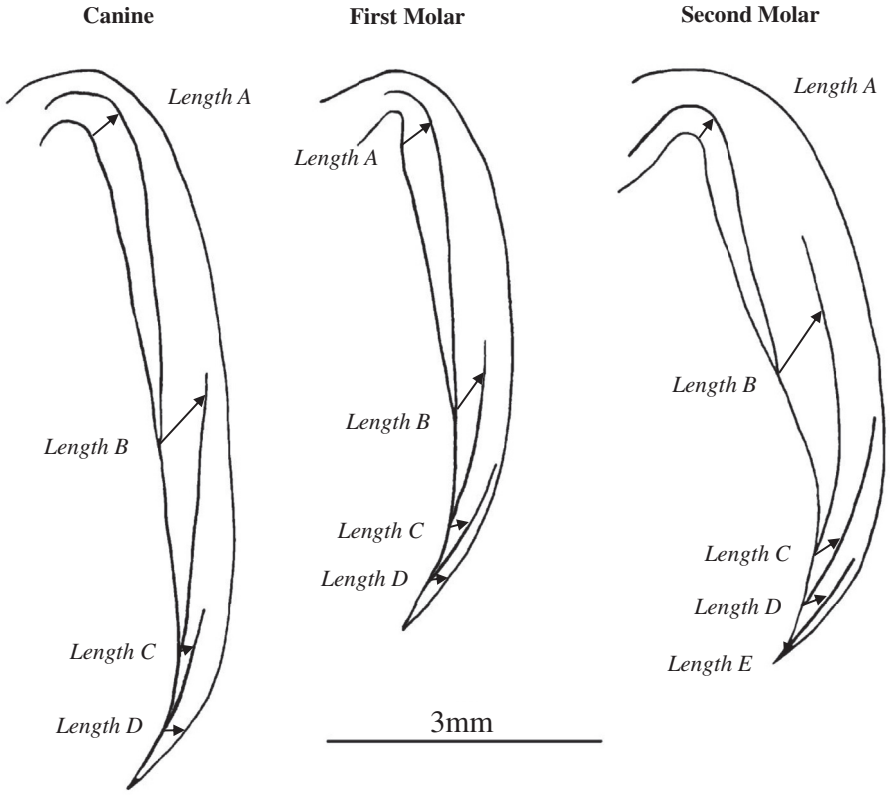

Fig. 2. Diagrams made using a drawing tube, to show how the crown formation times, before birth (Length A) and after birth (Lengths B–E) were measured in the buccal aspect of ten photomontages for each tooth type. Measurements were made from the EDJ along a prism path to the neonatal line or first accentuated striae, this neonatal line or striae was then traced back to the EDJ, where the process was repeated until the cervical point was reached. The sum of the enamel formation times calculated for each of these measurements is equal to the total enamel formation time.

Table 1a
This table shows the independent sample *t*-tests calculated to identify any significant differences between the two dental aspects in each region for each tooth type. Since three tests were performed for each tooth (one for each region), the *p*-value for the level of significance 0.05 was corrected to $p < 0.0167$ (Bonferroni correction) in order to avoid increased type 1 error frequency.[a]

Region	Central incisor							Lateral incisor							Canine				
	Aspects	Mean (μm)	Std. deviation	Std. error mean	95% Confidence interval of the difference (μm)		Significance (2-tailed)	Aspects	Mean (μm)	Std. deviation	Std. error mean	95% Confidence interval of the difference (μm)		Significance (2-tailed)	Aspects	Mean (μm)	Std. deviation	Std. error mean
					Lower	Upper						Lower	Upper					
Cervical	Labial Lingual ($N = 4$)	1.250	1.258	0.629	−0.752	3.252	0.141	Labial Lingual ($N = 5$)	0.400	2.702	1.208	−2.955	3.755	0.757	Labial Lingual ($N = 6$)	−0.333	2.160	0.882
Lateral	Labial Lingual ($N = 7$)	1.857	2.410	0.911	−0.372	4.086	0.088	Labial Lingual ($N = 7$)	1.000	2.309	0.873	−1.136	3.136	0.296	Labial Lingual ($N = 15$)	1.333	5.367	1.386
Occlusal	Labial Lingual ($N = 8$)	0.750	1.832	0.648	−0.782	2.282	0.285	Labial Lingual ($N = 9$)	1.222	3.866	1.289	−1.749	4.194	0.371	Labial Lingual ($N = 14$)	−3.000	7.514	2.008

N = number of paired aspects.
[a] The *p*-value is a number between 0 and 1 that reflects the strength of the data that are being used to evaluate the null hypothesis. If the *p*-value is small, then there is strong evidence against the null hypothesis, while a large *p*-value indicates weak evidence against the null hypothesis.

striations between the different regions on the same dental aspect. Only the first and second molars showed any significant differences between the different regions of the same dental aspect, this was apparent in the lateral and occlusal regions of both molars. In the first molar there was a significant difference on the lingual aspect between the lateral and occlusal regions but not between these regions on the buccal aspect. In the second molar there was no significant difference on the buccal or lingual aspect between the cervical and lateral regions or between the cervical and occlusal regions. There was, however, a significant difference between the lateral and occlusal regions on both aspects.

Unfortunately, the sample size was too small (i.e. less than 5) in the cervical/lateral and cervical/occlusal comparisons of the labial aspect of the central incisor and in the cervical/occlusal comparison of the lingual aspect of the lateral incisor, to obtain an accurate measure of statistical significance. However, as the other regions on the same aspect of both of these teeth showed no significance it seems reasonable to suggest that the whole tooth grew enamel in the same manner with no discernible differences between regions on the same aspect.

Based on the above results, all three regions on the labial and lingual aspects of the central and lateral incisors and of the canines were treated as being identical. The data for each region were therefore combined for each tooth type in subsequent analyses. In the case of the first molar the combined data for all regions of the buccal aspect and the cervical region on the lingual aspect were used in subsequent analyses. For the second molar the statistical tests revealed that the *inner* rates of enamel formation were not significantly different from each other between regions of the crown. However, when the longer trajectories of the occlusal and lateral enamel were compared, the diverging rates of outer enamel formation caused the occlusal and lateral regions to become significantly different. As a result of this it was decided to exclude the occlusal data completely and to combine the lateral and cervical data to generate a regression formula to predict enamel formation times in the second molar. A condition of doing this was that enamel prism lengths longer than those in the lateral enamel could not be used in subsequent predictions of enamel formation times in the occlusal region. However, in practice, no lengths beyond the inner enamel were ever measured in second molars (or in any other tooth type) in this study.

Fig. 3 shows the regression plots for each tooth type with their corresponding regression lines. These plots were generated from the combined cumulative cross-striation counts that were made

Table 1b
This table shows the paired sample *t*-tests calculated to identify any significant differences between the three regions on the same dental aspect for each tooth type.

Aspect	Central incisor							Lateral incisor							Canine			
	Regions	Mean (μm)	Std. deviation	Std. error mean	95% Confidence interval of the difference (μm)		Significance (2-tailed)	Regions	Mean (μm)	Std. deviation	Std. error mean	95% Confidence interval of the difference (μm)		Significance (2-tailed)	Regions	Mean (μm)	Std. deviation	Std. error mean
					Lower	Upper						Lower	Upper					
Labial/buccal	Cervical Lateral ($N = 4$)	0.250	1.258	0.629	−1.752	2.252	0.718	Cervical Lateral ($N = 6$)	−1.667	4.227	1.726	−6.103	2.769	0.378	Cervical Lateral ($N = 6$)	2.000	3.521	1.438
Lingual	Cervical Lateral ($N = 6$)	0.000	1.414	0.577	−1.484	1.484	1.000	Cervical Lateral ($N = 5$)	0.800	1.304	0.583	−0.819	2.419	0.242	Cervical Lateral ($N = 7$)	4.286	4.716	1.782
Labial/buccal	Cervical Occlusal ($N = 4$)	0.750	1.500	0.750	−1.637	3.137	0.391	Cervical Occlusal ($N = 6$)	0.333	4.676	1.909	−4.574	5.241	0.868	Cervical Occlusal ($N = 6$)	3.333	4.033	1.647
Lingual	Cervical Occlusal ($N = 6$)	0.000	2.098	0.856	−2.201	2.201	1.000	Cervical Occlusal ($N = 4$)	2.250	4.113	2.056	−4.295	8.795	0.354	Cervical Occlusal ($N = 7$)	1.000	2.309	0.873
Labial/buccal	Lateral Occlusal ($N = 9$)	1.667	2.179	0.726	−0.009	3.342	0.051	Lateral Occlusal ($N = 13$)	2.000	3.162	0.877	0.089	3.911	0.042	Lateral Occlusal ($N = 21$)	2.905	5.726	1.250
Lingual	Lateral Occlusal ($N = 7$)	0.429	1.718	0.649	−1.161	2.018	0.534	Lateral Occlusal ($N = 8$)	2.250	2.252	0.796	0.367	4.133	0.026	Lateral Occlusal ($N = 15$)	−1.000	8.150	2.104

N = number of paired regions.

Canine			First molar							Second molar						
95% Confidence interval of the difference (μm)		Significance (2-tailed)	Aspects	Mean (μm)	Std. deviation	Std. error mean	95% Confidence interval of the difference (μm)		Significance (2-tailed)	Aspects	Mean (μm)	Std. deviation	Std. error mean	95% Confidence interval of the difference (μm)		Significance (2-tailed)
Lower	Upper						Lower	Upper						Lower	Upper	
−2.600	1.934	0.721	Buccal Lingual ($N = 11$	4.000	4.899	1.477	0.709	7.291	0.022	Buccal Lingual ($N = 4$)	−1.000	5.354	2.677	−9.520	7.520	0.734
−1.639	4.306	0.352	Buccal Lingual ($N = 28$)	6.929	7.328	1.385	4.087	9.770	0.000	Buccal Lingual ($N = 30$)	−1.433	4.523	0.826	−3.122	0.256	0.093
−7.339	1.339	0.159	Buccal Lingual ($N = 20$)	5.300	4.835	1.081	3.037	7.563	0.000	Buccal Lingual ($N = 27$)	−0.519	6.818	1.312	−3.216	2.179	0.696

along the prism paths taken from the photomontages of the deciduous enamel ground sections. In all cases R^2 was greater than 0.9 and in no case was it improved by fitting a polynomial regression curve to the data. The regression equation for each tooth type is also presented alongside each plot with X being the enamel thickness (i.e. length of the enamel prism). These equations were used as the basis for predicting enamel formation time (days) from enamel thickness measurements (μm) taken from the EDJ to the enamel surface.

The regression formulae were used to produce a reference table of predicted enamel formation times for each tooth type (Table 2). This table shows that the number of days taken to form small lengths of enamel prism near the EDJ are initially very similar for all tooth types. Indeed, even the upper and lower 95% confidence limits are only a few days different to each of the mean predictions. However, as enamel thickness increases, bigger differences begin to emerge between each tooth type. At, for example, a prism length of 800 μm in deciduous canines and in first and second molars, the predictions for the number of days to form this length of prism are 210, 206 and 231 days respectively. These observations suggest first, that more accurate predictions are likely for shorter rather than longer prism lengths from the EDJ, but they also confirm that the enamel growth trajectories (the slopes of the plots) for different tooth types are indeed slightly different to one another. This becomes more important were one to use these regression formulae to predict the time taken to form longer lengths of enamel prism in different deciduous tooth types. Clearly though, these regression formulae cannot be used to predict the time taken to form greater thicknesses of enamel than those used here to generate the formulae.

The regression formulae were also applied to the photomontages of a second sample of ten ground sections of each tooth type ($n = 50$). In the majority of these sections a clear neonatal line is visible, allowing both pre- and postnatal enamel as well as the total crown formation times to be estimated along with the proportions of enamel formed before and after birth. Table 3 shows these enamel formation times expressed in days, weeks and months, as well as indicating the error margin in days (rounded to the nearest decimal place) and the percentage proportion of pre- and postnatal enamel formed for each tooth type. These results were added to the bottom of three tables of data collated from the previously published literature in order to enable a comparison of the results (see Tables 4–6).

From the use of these formulae, enamel initiation and completion times for this second sample of deciduous teeth were established. These estimates of deciduous crown formation times and the amount of enamel present at birth corresponded well with the previously published estimates (see Tables 4–6).

The histological method outlined in this study has been used to update the previous estimates of deciduous crown formation times without the use of fetal material and therefore without the errors encountered when using this material. In the majority of longitudinal sections a neonatal line is usually visible in deciduous enamel, so it is possible to estimate both prenatal and postnatal enamel formation times and also to calculate the proportions of each for each tooth type. The regression equations generated here could also be used to estimate deciduous enamel formation times in developing teeth and to provide an age at death in infants less than ~1 year old (where enamel formation has ceased prior to deciduous enamel completion). In order to investigate this further, a

Canine			First molar							Second molar						
95% Confidence interval of the difference (μm)		Significance (2-tailed)	Regions	Mean (μm)	Std. deviation	Std. error mean	95% Confidence interval of the difference (μm)		Significance (2-tailed)	Regions	Mean (μm)	Std. deviation	Std. error mean	95% Confidence interval of the difference (μm)		Significance (2-tailed)
Lower	Upper						Lower	Upper						Lower	Upper	
−1.695	5.695	0.223	Cervical Lateral ($N = 10$)	−0.800	3.259	1.031	−3.131	1.531	0.458	Cervical Lateral ($N = 5$)	0.600	4.722	2.112	−5.263	6.463	0.790
−0.076	8.647	0.053	Cervical Lateral ($N = 10$)	−0.200	3.425	1.083	−2.650	2.250	0.858	Cervical Lateral ($N = 7$)	1.286	4.071	1.539	−2.479	5.051	0.435
−0.899	7.566	0.099	Cervical Occlusal ($N = 10$)	−0.700	4.762	1.506	−4.107	2.707	0.653	Cervical Occlusal ($N = 5$)	0.800	4.604	2.059	−4.917	6.517	0.717
−1.136	3.136	0.296	Cervical Occlusal ($N = 10$)	−0.200	3.824	1.209	−2.935	2.535	0.872	Cervical Occlusal ($N = 7$)	3.857	4.947	1.870	−0.718	8.433	0.085
0.298	5.511	0.031	Lateral Occlusal ($N = 23$)	3.348	7.444	1.552	0.129	6.567	0.042	Lateral Occlusal ($N = 29$)	4.862	8.564	1.590	1.605	8.120	0.005
−5.514	3.514	0.642	Lateral Occlusal ($N = 23$)	2.913	4.542	0.947	0.949	4.877	0.006	Lateral Occlusal ($N = 24$)	4.375	4.312	0.880	2.554	6.196	0.000

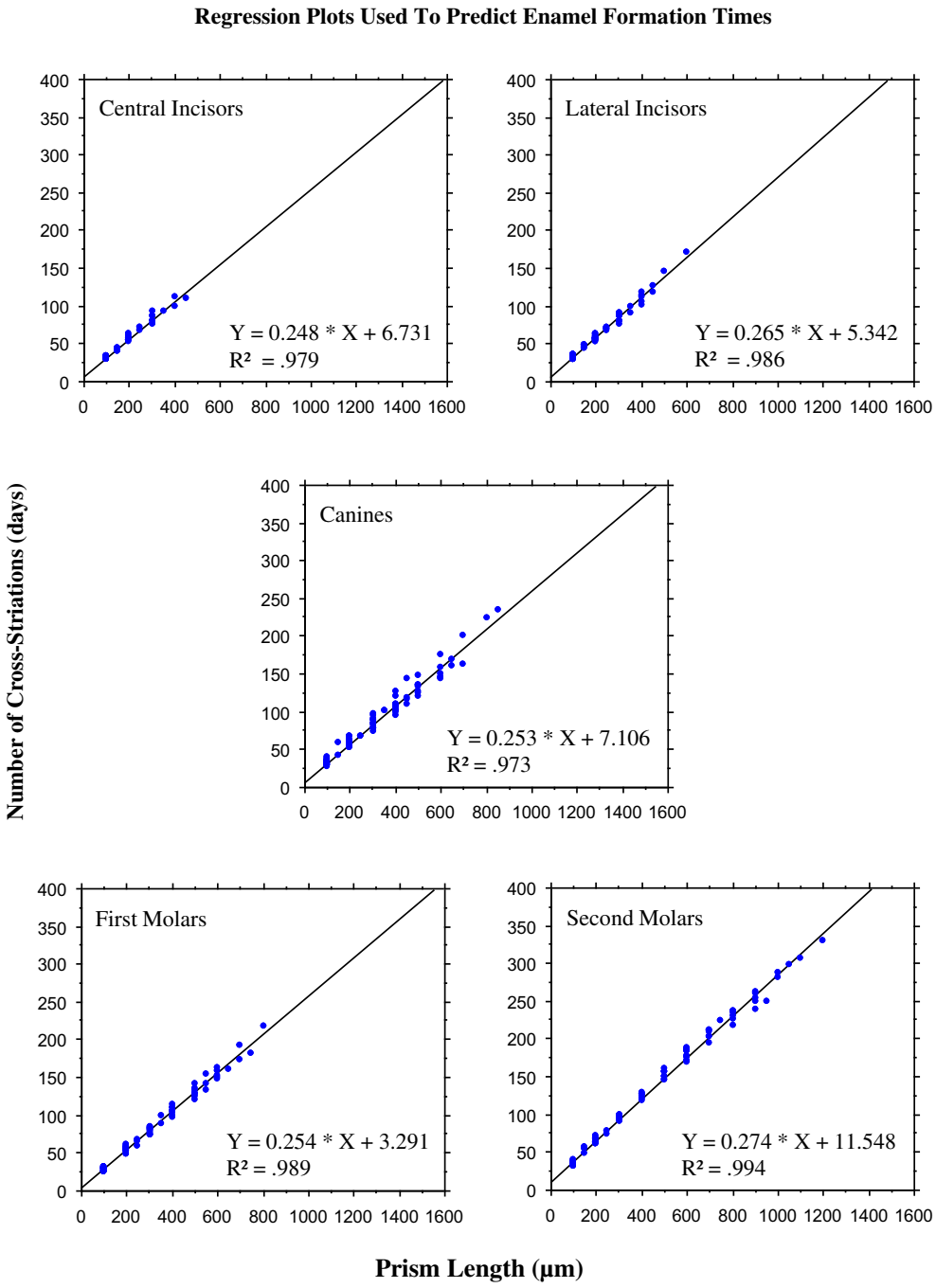

Fig. 3. Regression plots for each tooth type with their corresponding regression lines. The linear regression equation for each tooth type is also presented alongside each plot with X being the enamel thickness (i.e. length of the enamel prism from the EDJ to the enamel surface). These equations were used to predict enamel formation times (days) from enamel prism length measurements (μm) made from the EDJ.

blind test was carried out using deciduous teeth from two individuals with a known medical history (see below).

4. Discussion – part 1

It can be seen from Table 2 that 100 μm of enamel at the EDJ in central incisors form at an average rate of 3.12 μm per day (100 μm/mean number of days = 32 days) whereas at the outer enamel at 1000 μm in the thicker enamelled deciduous second molars this average is closer to 3.49 μm per day (1000 μm/mean number of days = 286 days). The estimates made here from the regression equations, however, in effect 'smooth out' many local fluctuations in the enamel formation rate in an individual tooth which are then not taken account of by the use of these equations. Notably, neither the decrease in the formation rate of approximately 0.5 μm per day that occurs immediately after the neonatal line,[19,20] or any other accentuated marking that may be specific to an individual tooth are accounted for by these regression formulae. It follows that the formation times they generate remain 'estimates' and will always be less reliable than when every daily increment in a tooth section is counted directly. However the use of these regression equations does provide a method of calculating deciduous crown formation times which can be used for analysing sections of either archaeological or forensic material where the daily cross-striations are

Table 2
Predicted enamel formation times in days for each 10 μm measurement of enamel prism length between 50 μm to 100 μm and then for each subsequent 50 μm thickness of enamel for each deciduous tooth type. These data were generated using the regression formulae, which give the mean time of enamel formation in days (Y) as well as the ranges within the upper and lower 95% confidence limits.

Central incisors

Prism length (μm)	Mean (days)	Confidence limits		Range (days)
		95% Lower (days)	95% Upper (days)	
50	19	17	22	2.6
60	22	19	24	2.7
70	24	21	27	2.8
80	27	24	29	2.9
90	29	26	32	3.0
100	32	28	35	3.1
150	44	40	47	3.6
200	56	52	60	4.1
250	69	64	73	4.6
300	81	76	86	5.1
350	94	88	99	5.6
400	106	100	112	6.1
450	118	112	125	6.6

Mean: $Y = 0.248 \times$ Prism Length (μm) + 6.731.
Lower 95% confidence limit: $Y = 0.238 \times$ Prism Length (μm) + 4.678.
Upper 95% confidence limit: $Y = 0.258 \times$ Prism Length (μm) + 8.784.

Lateral incisors

Prism length (μm)	Mean (days)	Confidence limits		Range (days)
		95% Lower (days)	95% Upper (days)	
50	19	16	21	2.2
60	21	19	23	2.2
70	24	22	26	2.3
80	27	24	29	2.4
90	29	27	32	2.4
100	32	29	34	2.5
150	45	42	48	2.9
200	58	55	62	3.2
250	72	68	75	3.6
300	85	81	89	3.9
350	98	94	102	4.3
400	111	107	116	4.6
450	125	120	130	5.0
500	138	133	143	5.3
550	151	145	157	5.7
600	164	158	170	6.0

Mean: $Y = 0.265 \times$ Prism Length (μm) + 5.342.
Lower 95% confidence limit: $Y = 0.258 \times$ Prism Length (μm) + 3.535.
Upper 95% confidence limit: $Y = 0.272 \times$ Prism Length (μm) + 7.148.

Canines

Prism length (μm)	Mean (days)	Confidence limits		Range (days)
		95% Lower (days)	95% Upper (days)	
50	20	16	23	3.4
60	22	19	26	3.5
70	25	21	28	3.6
80	27	24	31	3.7
90	30	26	34	3.8
100	32	29	36	3.9
150	45	41	49	4.3
200	58	53	62	4.8
250	70	65	75	5.2
300	83	77	88	5.7
350	96	90	101	6.1
400	108	102	114	6.6
450	121	114	128	7.0
500	134	126	141	7.5
550	146	138	154	7.9
600	159	151	167	8.4
650	172	163	180	8.8
700	184	175	193	9.3
750	197	187	206	9.7
800	210	199	219	10.2
850	222	212	232	10.6

Mean: $Y = 0.253 \times$ Prism Length (μm) + 7.106.

Table 2 (*continued*)

Canines

Prism length (μm)	Mean (days)	Confidence limits		Range (days)
		95% Lower (days)	95% Upper (days)	

Lower 95% confidence limit: $Y = 0.244 \times$ Prism Length (μm) + 4.123.
Upper 95% confidence limit: $Y = 0.261 \times$ Prism Length (μm) + 10.089.

First molars

Prism length (μm)	Mean (days)	Confidence limits		Range (days)
		95% Lower (days)	95% Upper (days)	
50	16	13	19	2.6
60	19	16	21	2.7
70	21	18	24	2.8
80	24	21	26	2.8
90	26	23	29	2.9
100	29	26	32	2.9
150	41	38	45	3.2
200	54	51	58	3.5
250	67	63	71	3.8
300	79	75	84	4.1
350	92	88	97	4.4
400	105	100	110	4.7
450	118	113	123	5.0
500	130	125	136	5.3
550	143	137	149	5.6
600	156	150	162	5.9
650	168	162	175	6.2
700	181	175	188	6.5
750	194	187	201	6.8
800	206	199	214	7.1

Mean: $Y = 0.254 \times$ Prism Length (μm) + 3.291.
Lower 95% Confidence Limit: $Y = 0.248 \times$ Prism Length (μm) + 0.951.
Upper 95% Confidence Limit: $Y = 0.260 \times$ Prism Length (μm) + 5.631.

Second molars

Prism length (μm)	Mean (days)	Confidence limits		Range (days)
		95% Lower (days)	95% Upper (days)	
50	25	23	28	2.6
60	28	25	31	2.7
70	31	28	33	2.7
80	33	31	36	2.8
90	36	33	39	2.8
100	39	36	42	2.9
150	53	50	56	3.1
200	66	63	70	3.4
250	80	76	83	3.6
300	94	90	97	3.9
350	107	103	111	4.1
400	121	117	125	4.4
450	135	130	139	4.6
500	149	144	153	4.9
550	162	157	167	5.1
600	176	171	181	5.4
650	190	184	195	5.6
700	203	197	209	5.9
750	217	211	222	6.1
800	231	224	236	6.4
850	244	238	250	6.6
900	258	251	264	6.9
950	272	265	278	7.1
1000	286	278	292	7.4
1050	299	292	306	7.6
1100	313	305	320	7.9
1150	327	319	334	8.1
1200	340	332	348	8.4

Mean: $Y = 0.274 \times$ Prism Length (μm) + 11.548.
Lower 95% Confidence Limit: $Y = 0.269 \times$ Prism Length (μm) + 9.194.
Upper 95% Confidence Limit: $Y = 0.278 \times$ Prism Length (μm) + 13.902.

often poorly preserved or invisible. The only requirements of the method described here are that in the ground sections examined, the direction of the enamel prisms is visible and there are sufficient accentuated markings (long-period striae) visible in the lateral

Table 3
Total enamel formation times (mean values given in days, weeks and months) estimated for a sample of ten ground sections of each tooth type. The percentage proportion of prenatal and postnatal enamel are shown together with the upper and lower 95% confidence limits for the estimates given in days.

		Mean (days)	% Crown Completion	Confidence limits		Range (days)	Weeks (mean day/7)	Months (mean day/30.44)
				95% Lower (days)	95% Upper (days)			
Central incisor	Crown formation time before birth	144	59.99%	137	152	15	20.6	4.74
	Crown formation time after birth	96	40.01%	89	103	15	13.7	3.16
	Total crown formation time	240	100%	226	255	30	34.4	7.90
Lateral incisors	Crown formation time before birth	136	54.81%	131	142	11	19.5	4.48
	Crown formation time after birth	113	45.19%	107	119	12	16.1	3.70
	Total crown formation time	249	100%	238	260	22	35.6	8.18
Canines	Crown formation time before birth	128	29.87%	121	135	14	18.4	4.22
	Crown formation time after birth	302	70.13%	280	322	42	43.1	9.91
	Total crown formation time	430	100%	401	458	56	61.4	14.13
First molars	Crown formation time before birth	140	43.01%	135	146	11	20.0	4.61
	Crown formation time after birth	186	56.99%	175	197	21	26.5	6.10
	Total crown formation time	326	100%	310	342	33	46.6	10.71
Second molars	Crown formation time before birth	118	23.23%	113	122	8	16.8	3.86
	Crown formation time after birth	389	76.77%	373	403	30	55.5	12.77
	Total crown formation time	506	100%	487	524	38	72.3	16.64

enamel in order to track enamel formation from the region of the dentine horn to the cervix.

The data generated from this new approach allows a number of areas of deciduous crown development to be revisited and reassessed, in particular the prenatal and postnatal enamel formation times for each tooth type. The neonatal line is recognised as an important biological landmark which can be used to precisely define prenatal and postnatal enamel (see Zanolli et al., 2011[23] for an up to date review on the nature and form of the neonatal line). The neonatal line makes it possible to establish the time of initial mineralisation for deciduous enamel in utero, without the need to work with fetal specimens and without encountering the major problem of determining an accurate age of the fetal specimens examined. Using the neonatal line as a biological landmark, the regression equations were applied to determine the amount of time taken to form both the pre- and postnatal enamel for each tooth type. The sum of these resultant times could therefore then provide the total crown formation times for each tooth type (see Table 3).

Initial crown mineralisation times for deciduous enamel can be established for each tooth type by using the prenatal enamel formation times established by the regression equations and calculating backwards from birth. This can be done by subtracting the mean number of days of prenatal enamel formation from the duration of an average pregnancy, which is 39 weeks for an average singleton birth.[24] The resultant mean average and the 95% confidence limits range for initial mineralisation obtained in this study are shown at the bottom of Table 4, along with the data collated from the literature in order to allow a comparison.

Deciduous enamel initiation and completion times have been established in the literature from a variety of methods and techniques, each of which has its advantages and disadvantages. A review of the literature of all the available enamel initiation times has been collated and these times are presented in Table 4. This data has been cited directly from the original sources, however some data, originally presented in 'months' or 'days', have been converted to 'weeks'. This conversion was performed to enable an easier comparison, however, in all cases the original data is presented in parentheses. Although a direct comparison of the ages at which deciduous teeth commence mineralisation presented by previous authors may not be possible, '*unless the results are converted to a common basis*', no attempt, however, has been made to convert this data to either 'fertilization age' as was done by Lunt and Law[11:604] or to 'post-menstrual age' as done by Sunderland et al.[12:173] This avoids further confusion, inconsistency and the occurrence of errors that have already been introduced by previous attempts to perform this conversion. Nonetheless, it is important to recognise that 'menstrual age' is two weeks longer than 'fertilization age' and so there is potentially a two week difference in the range of ages presented by different authors.

From this review of the literature all of the available enamel completion times were also collated and these are presented in Table 5 as well as the proportion of enamel thickness present at birth (Table 6).

As illustrated by Table 4, the previous studies reviewed show considerable variation in the initial mineralisation times for individual teeth. The data show little consistency, varying from Robin and Magitot (1860–63) at the lower end of the range to Meyer[25] at the upper end. As McCall and Wald[26:96] pointed out, until the work of Logan, Schour and colleagues, prenatal dental development had received relatively little attention from researchers. '*The basic work of early investigators established, to the satisfaction of all for nearly two generations, the modus operandi and chronology*' of prenatal dental development. McCall and Wald also added '*that it seems to have been taken for granted by many that no important additional information was to be gained regarding prenatal tooth development*'. This long tradition of not questioning the 'current' dental chronologies has meant that errors have been perpetuated throughout the literature of deciduous enamel formation; this appears to have been the case until the works of Logan, Kronfeld and Schour.[13,14,27–29] However, following this spate of research a second void in studies of prenatal dental developmental research then occurred and persisted until the work of Kraus[30,31] and Kraus and Jordan[8] was published. Finally in 1974 Lunt and Law suggested that the mineralisation table produced by Kronfeld and Schour[28] be updated, it was only at this time that the various chronologies of deciduous enamel formation were revisited in an objective way. It can be concluded from the literature review that many general statements have been made regarding mineralisation of the deciduous dentition and that these have often been presented without adequate evidence or critical evaluation. In some cases statements have been presented as facts without reference to the original sources. In this way errors have been perpetuated in the literature and rarely challenged.

When compared to the data collated from the literature detailing crown initiation times, not surprisingly the initiation times

Table 4
Initial mineralisation table showing data collated from the literature in chronological order expressed in gestational weeks.[7–12,14,17,18,25,27–30,32–42] The mineralisation data resulting from this study have been added for comparison.[a]

Author	Date of publication	Central incisor		Lateral incisor		Canine		First molar		Second molar	
		Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular
Robin & Magitot	1860–63	–	11.43–12.14 (80–85 days)	–	13.43–14.14 (94–99 days)	–	17.14–17.86 (120–125 days)	–	12.43–13.14 (87–92 days)	–	15.43–16.28 (108–114 days)
Peirce	1877	17		17		17		18		18	
Legros & Magitot	1880	16	16	16	16	16 in text. 17 in table.	16	17	17	17	17
Peirce	1884	17		17		17		18 in chart. 19 in text.		18 in chart. 19 in text.	
Tomes	1889	17		17		17		18		18	
Broomell & Fischelis	1913	16 (4 mths)	16 (4 mths)	16 (4 mths)	16 (4 mths)	20 (5 mths)	20 (5 mths)	20 (5 mths)	20 (5 mths)	20–24 (5–6 mths)	20–24 (5–6 mths)
Tomes	1914	20		20		24		24		24	
Mummery	1924	20		20		24		24		24	
Brady	1924	17		17		17		20		20	
Churchill	1932	18 (4.5 mths)		18 (4.5 mths)		22 (5.5 mths)		20 (5 mths)		22 (5.5 mths)	
Wolfe	1935	17 in text. 20 in chart.		17 in text. 20 in chart.		24		24		24	
Meyer	1935	20 (5 mths)		20 (5 mths)		24 (6 mths)		20 (5 mths)		32 (8 mths)	
Kronfeld	1935 & 1937	20 (5 mths)		20 (5 mths)		24 (6 mths)		20 (5 mths)		24 (6 mths)	
Schour & Kronfeld	1938	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		22 (5.5 mths)	
Kronfeld & Schour	1939	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		24 (6 mths)	
Schour & Massler	1940	16 (4 mths)	18 (4.5 mths)	18 (4.5 mths)		20 (5 mths)		20 (5 mths)		24 (6 mths)	
Kraus	1959	12–16		–		–		–		14–22	
Turner	1963	–		–		–		18		19–20	
Nomata	1964	17	17.66 ($17^2/_3$)	19.66 ($19^2/_3$)	17.66 ($17^2/_3$)	21.33 ($21^1/_3$)	19.66 ($19^2/_3$)	19.33 ($19^1/_3$)	19.66 ($19^2/_3$)	21.33 ($21^1/_3$)	23.66 ($23^2/_3$)
Kraus & Jordan	1965	14		16		17		15.5		19	18
Lunt & Law (average)	1974	14		16		17		15.5 (15½)		19	18
Lunt & Law (range)	1974	13–16		14.66–16.5 ($14^2/_3$–16½)	14.66 ($14^2/_3$)	15–18	16	14.5 (14½)–17		16–23.5 (23½)	17–19.5 (19½)
Sunderland et al.	1987	15–19		16–21		19–22		16–19		20–22	
Mahoney	2011	–		–		–		–	19–26	–	25–31
Birch (average)[b]	2011	–	18	–	20	–	21	–	19	–	22
Birch (range)[c]	2011	–	17–19	–	19–20	–	20–22	–	18–20	–	22–23

[a] Original data are given in parentheses and were converted to weeks for comparison. Where information was available mandibular and maxillary teeth are presented separately.
[b] 273 days (39 weeks) - mean CFT before birth (days)/7.
[c] 273 days (39 weeks) - 95% confidence limits (days)/7.

Table 5
Crown completion table showing data collated from the literature in chronological order, expressed in weeks after birth. The mineralisation data resulting from this study have been added for comparison.[a]

Author	Date of publication	Form of original data	Type of conversion	Central incisor		Lateral incisor		Canine		First molar		Second molar	
				Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular
Broomell & Fischelis	1913	Months	Months × 4	8 (2 mths)		8 (2 mths)		8 (2 mths)		8 (2 mths)		8 (2 mths)	
Meyer	1935	Months	Months × 4	12 (3 mths)		12 (3 mths)		48 (1 year)		36 (9 mths)		–	
Kronfeld	1935 & 1937	Months	Months × 4	16 (4 mths)		20 (5 mths)		36 (9 mths)		24 (6 mths)		40–48 (10–12 mths)	
Kronfeld & Schour	1939	Months	Months × 4	6 (1.5 mths)	10 (2.5 mths)	10 (2.5 mths)	12 (3 mths)	36 (9 mths)	36 (9 mths)	24 (6 mths)	22 (5.5 mths)	44 (11 mths)	40 (10 mths)
Schour & Massler	1940	Months	Months × 4	6 (1.5 mths)	10 (2.5 mths)	10 (2.5 mths)	12 (3 mths)	36 (9 mths)	36 (9 mths)	24 (6 mths)	22 (5.5 mths)	44 (11 mths)	40 (10 mths)
Lunt & Law	1974	Months	Months × 4	6 (1.5 mths)	10 (2.5 mths)	10 (2.5 mths)	12 (3 mths)	36 (9 mths)	36 (9 mths)	24 (6 mths)	22 (5.5 mths)	44 (11 mths)	40 (10 mths)
Mahoney	2011	Days	Days/7	–		–		–		–	39	–	56
Birch (average)	2011	Days	Mean days/7	–	13	–	16	–	43	–	26	–	55
Birch (range)	2011	Days	Mean days/7	–	12–15	–	15–17	–	40–46	–	25–28	–	53–58

[a] Original data are given in parentheses and were converted to weeks for comparison. Where information was available mandibular and maxillary teeth are presented separately.

obtained using the regression formulae fall within the range of those reported in the literature (Table 4), this is because the range in this data set is so large. However, when compared to the results obtained using 'tooth ring analysis' which is the nearest comparable histological method of study,[14,28,29] the results are somewhat similar. Except for the lateral incisor (which is one week later than that obtained by 'tooth ring analysis') and the second molar (which is one week earlier than that obtained by 'tooth ring analysis'), the results obtained by 'tooth ring analysis' fall within the range established by this study. What is interesting is that the time of 22 weeks, which was obtained using the regression formulae, corresponds with the 1938 study of Schour and Kronfeld but not with the 1939 study of Kronfeld and Schour, where a discrepancy of one week occurs. Unfortunately, no explanation of why the original time was increased to 24 weeks by Kronfeld and Schour[28] is given. Recent work by Mahoney,[10] who investigated the incremental structure of deciduous mandibular molars, presented mean prenatal formation times of 113 days for first molars and 74 days for second molars. When compared to the times obtained from this study, for the first molar there is a discrepancy of 27 days from the mean of 140 days and for the second molar there is a discrepancy of 44 days from the mean of 118 days. Mahoney also presented a range of 49 days for the first molar and of 42 days for the second molar (see Table 4), which seems excessive when compared to the range in this study of 11 days for the first and 8 days for the second molar (see Table 3). It is possible that these differences result simply from sampling different populations of teeth (modern and archaeological) or from very small sample sizes, but they might also in theory result from different degrees of section obliquity. Section obliquity is one limitation of estimating the period of prenatal enamel formation that can lead to inaccuracy. Very small shifts in the plane of section have large effects on measurements of the true linear distance between the tip of the dentine horn and the neonatal line. This is especially so in the case of deciduous teeth where such small and often pointed cusps make it very hard to produce ground sections in the true plane of section.

As with the prenatal enamel formation times, it was only after the identification and confirmation that the neonatal line did in fact have a neonatal origin that it became possible to accurately determine the precise difference between pre- and postnatal enamel. Again using the neonatal line as a biological landmark, the regression equations were applied to determine the amount of time taken to form the postnatal enamel for each tooth type. The resultant mean average and the 95% confidence limits range for postnatal mineralisation are shown in Table 5, along with the data collated from the literature review included for comparison.

When compared to the data collated from the literature, the postnatal times obtained using the regression formulae, unlike the initiation times, do not correspond well with those reported previously. The average results obtained in this study increase the time of crown formation from a minimum of three weeks to a maximum of 15 weeks from the previously recorded times obtained by 'tooth ring analysis'. The greatest discrepancy being in the second molars which Kronfeld and Schour[28] and Schour and Massler[29] stated complete at 40 weeks, however the results obtained in this study increase this completion time to 53–58 weeks. The recent work by Mahoney[10] presented postnatal formation times of 275 days for first molars and 396 days for second molars. The mean values obtained in this study are 186 and 389 respectively. While the mean for the first molar is 89 days different, those for the second molar are within seven days of the same value and compare well with those of Mahoney. Again it is possible that these differences result simply from observing very small sample sizes or are due to differences in section obliquity or sample source, i.e. modern compared to archaeological. However, for the second molar there is

Table 6
Proportion of crown completed at birth. Data derived from the literature review presented in chronological order expressed as a percentage of total enamel formation. The mineralisation data resulting from this study have been added for comparison.

Author	Date of publication	Central incisor		Lateral incisor		Canine		First molar		Second molar	
		Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular	Maxillary	Mandibular
Peirce	1884	'Quite complete'		'Quite complete'		66.66% (2/3)		66.66% (2/3)		50% (1/2)	
Mummery	1924	'Crowns calcified'		'Crowns calcified'		'Tips calcified'		'Cusps united'		'Cusps united'	
Churchill	1932	50% (1/2)		40% (2/5)		25% (1/4)		'Cusps united'		20% (1/5)	
Hess [43] et al.	1932	66.66% (2/3)		66.66% (2/3)		25% (1/4)		'Little more than the occlusal surface'		'Base of the cusps' 'incompletely calcified'	
Meyer	1935	'Almost complete'		'Half complete'		–		–		'Crown tips have coalesced'	
Kronfeld & Schour	1939	83.33% (5/6)	60% (3/5)	66.66% (2/3)	60% (3/5)	33.33% (1/3)		'Cusps united'		'Cusp tips still isolated'	
Schour & Massler	1940	83.33% (5/6)	60% (3/5)	66.66% (2/3)	60% (3/5)	33.33% (1/3)		'Cusps united'		'Cusp tips still isolated'	
Lunt & Law	1974	83.33% (5/6)	60% (3/5)	66.66% (2/3)	60% (3/5)	33.33% (1/3)		'Cusps united; occlusal completely calcified plus half to three fourths crown height'	'Cusps united; occlusal completely calcified'	'Cusps united; occlusal incompletely calcified; calcified tissue covers a fifth to a fourth crown height'	'Cusps united; occlusal incompletely calcified'
Mahoney	2011	–		–		–		–	29%	–	16%
Birch	2011	–	59.99%	–	54.81%	–	29.87%	–	43.01%	–	23.23%

Original data are given in parentheses and where information was available mandibular and maxillary teeth are presented separately.

a discrepancy of only seven days between these two studies from the mean of 389 days.

The total deciduous enamel formation times (the sum of prenatal and postnatal enamel formation times) can be established for each tooth type, and are presented in Table 3. Schour and Massler[29:1925] claimed that the canine '*consumes the longest time because of the length of its crown*', but in this study total enamel formation of the second molar (16.64 months) exceeded the canine (14.13 months). Mahoney[10] reported total crown formation times in an archaeological sample for the first molar as 388 days and for the second molar as 470 days, values that are close to those obtained in this study (326 days and 506 days respectively).

The proportion of enamel present at birth for each tooth type was difficult to compare with those determined in previous studies; this is due to the fact that many of the historical studies do not present quantitative data for this. Where it has been possible to present these data as percentages, the amounts appear to be similar to those obtained in this study. These are presented in Table 6. The largest discrepancy between this study and the previous 'tooth ring analysis' studies (6%) is in the lateral incisor.

In the future, larger sample sizes will undoubtedly both improve the accuracy with which these formulae can be used and also explore the possibility that differences in enamel formation rates may exist. For example, larger sample sizes may be able to identify differences between sex, geographical regions worldwide and/or between individuals of different socioeconomic backgrounds as well as confirm differences between modern and archaeological populations.

The data presented in this study are derived from a comparatively small sample of human deciduous teeth. Nonetheless, the results are consistent enough to suggest that it is indeed possible to use these data to establish crown formation times for deciduous teeth from other ground sections at defined confidence limits. This in effect enables longitudinal growth data to be retrieved from the incremental structure of enamel and could also permit the estimation of the chronological age for juveniles from their developing enamel crowns. In order to explore the suggestion that the regression equations could be used to obtain such information regarding growth they were applied to the teeth of two individuals with a known medical history.

5. Application of regression equations to two cases studies

In order to test the use of the regression formulae developed above as a method of age estimation that could be used in forensic anthropology, the equations were applied to ground sections of two exfoliated deciduous first mandibular molars from a pair of twins with a known medical history. These medical histories were consulted after the data had been obtained from the ground sections.

Although some striae were clearer on the lingual aspects, the buccal aspect was examined as this aspect is thicker and so contains the greatest number of increments of growth from initiation at the EDJ until the end of enamel formation at the buccal cervix. Therefore, in a forensic context, the buccal aspect is of more use when trying to establish an estimated age of an individual, as it potentially offers a longer time line than the lingual enamel. The buccal aspect was also shown to be more statistically constant during the analysis of the regression formulae.

Photomontages were produced of the ground sections from these individuals, (Twin A and Twin B). These montages were constructed from a series of overlapping photographic prints taken with an Olympus OM-2N camera loaded with Kodak Gold 200 film attached to a Carl Zeiss Jenamed 2 light microscope with an apochromat 25×/0.65 ∞/0.17-A objective lens. The resulting fieldwidth of a 5 × 7 inch print was 410 μm. Photomontages were constructed of

the occlusal region where the prenatal crown formation times had been previously calculated from whilst developing the formulae (see Length A in Fig. 2) and also slightly lower in the occlusal region where the postnatal enamel exhibited the clearest accentuated striae. This process was repeated for each twin.

In order to ascertain the time taken between each accentuated striae the methods used previously were adapted. Once the photomontages had been constructed, a sheet of clear acetate was placed over each photomontage and secured in place with 'Sellotape'. The positions of the accentuated striae were then traced onto the acetate using a fine-tipped permanent Staedtler Lumocolor pen. Care was taken to make constant reference to the ground section whilst this was being done in order to ensure that the striae were accurately identified on the photomontage and so correctly traced onto the acetate.

A straight line was drawn onto the acetate running in the general prism direction; care was taken to position the line along the majority of the prism path from its beginning at the EDJ to its termination at the enamel surface. This line was placed in the same location as before when calculating the enamel formation times. The length of the prism from the EDJ to the location where the neonatal line/accentuated striae crossed this line was recorded, each time recording the measurement from the EDJ to the point where the line first encountered each accentuated striae. Each measurement was repeated three times and the mean was recorded on the acetate.

The mean prism length recorded from the EDJ to each subsequent accentuated striae was then substituted into the appropriate regression formulae. Prenatal and postnatal enamel formation times were calculated in this way for both of the buccal aspects for each twin.

Another clear acetate sheet was then placed on top of the first acetate with the traced accentuated striae and again secured in place with 'Sellotape'. Where visible, daily cross-striation counts were recorded between each of the subsequent accentuated striae, commencing from the EDJ to the enamel surface following the course of the straight line. Each cross-striation was carefully marked onto the acetate with a fine-tipped permanent Staedtler Lumocolor pen, allowing this count to be verified and double checked. Where cross-striations were not always visible along the length of the same prism, the adjacent prism was used and the counts were transcribed onto this prism. When the use of a neighbouring prism was unavoidable, great care was taken not to add or subtract increments and so introduce errors into the final count. The number of counts of the daily increments at the point where the prism first encountered each accentuated striae was recorded.

The results obtained from the application of the regression formulae and the daily cross-striation counts were tabulated and then compared to each medical history.

6. Results

In order to ascertain the time between each accentuated striae, the regression formulae developed in the previous section, were applied to measurements taken from the photomontages of ground sections from the deciduous first molars of two twins. Daily cross-striation counts were also recorded for comparison.

6.1. Prenatal enamel

The results obtained for the prenatal formation times can be found in Table 7. This includes the predicted times derived from the

Table 7
Predicted mean results of the combined regression formulae when applied to the prenatal enamel of two ground sections of the deciduous first molar teeth from two individuals.[a]

Measurements from photomontage					Confidence limits		
Measurement	Prism length (mm)	Magnification factor (mm)	Prism length (μm)	Mean (days) predicted	95% Lower (days)	95% Upper (days)	Corresponding cross-striation direct counts
Twin A							
A	27.22	2.24	60.95	19	16	21	18
B	36.16	2.24	81.00	24	21	27	23
C	46.35	2.24	103.82	30	27	33	28
D	53.92	2.24	120.78	34	31	37	32
E	63.32	2.24	141.84	39	36	43	38
F	71.87	2.24	160.99	44	41	47	43
G	80.47	2.24	180.25	49	46	52	48
H	89.51	2.24	200.50	54	51	58	52
I	97.49	2.24	218.38	59	55	62	56
J	105.32	2.24	235.92	63	59	67	62
K	114.27	2.24	255.96	68	64	72	66
L	131.15	2.24	293.78	78	74	82	75
Twin B							
A	25.71	2.24	57.59	18	15	21	17
B	35.55	2.24	79.79	24	21	26	22
C	49.19	2.24	110.19	**31**	28	34	**31**
D	62.86	2.24	140.81	39	36	42	38
E	70.03	2.24	156.87	43	40	46	41
F	76.86	2.24	172.17	47	44	50	46
G	89.66	2.24	200.84	54	51	58	53
H	105.13	2.24	235.49	63	59	67	60
I	116.14	2.24	260.15	69	65	73	66
J	131.41	2.24	294.36	78	74	82	74

Mean: Enamel Formation Time (days) = 0.254 × Prism Length (μm) + 3.291.
Lower 95% Confidence Limit: Enamel Formation Time (days) = 0.248 × Prism Length (μm) + 0.951.
Upper 95% Confidence Limit: Enamel Formation Time (days) = 0.260 × Prism Length (μm) + 5.631.

[a] The crown formation times are expressed in days, as well as indicating the 95% confidence limits (rounded to the nearest decimal place). The cross-striation counts for each corresponding region are also presented to allow direct comparison of the two methods. Bold mean numbers correspond exactly between the mean regression formulae counts and the direct daily counts.

formulae and the corresponding daily cross-striation counts. The prenatal crown formation times for both twins calculated using the regression formulae resulted in 78 days of prenatal enamel formation for each individual; the daily cross-striation counts were very similar being 75 days for Twin A and 74 days for Twin B. Only on one occasion in Twin B did the mean formulae results and the daily counts match exactly (shown in bold in Table 7). However, there is only a discrepancy of four days maximum between the mean formulae results and the daily cross-striation counts and on no occasion do the corresponding direct counts fall outside of the range of the 95% confidence limits.

These prenatal enamel formation times appear to be much shorter than the prenatal formation times calculated in the previous section for first molars (see Table 3). This suggests that these twins may have been born prematurely. If the prenatal enamel formation time (78 days) is subtracted from the first molar crown formation time previously calculated (140 days with a range of 135–146 days), this infers that the twins were born 62 days prematurely.

The prenatal enamel found in both of these molars is unlike the prenatal enamel that was examined previously; this enamel appears to have accentuated striae approximately every four to seven days until the neonatal line is encountered. On average for both twins this is every 5.95 days. All of these accentuated striae are similar in appearance and although faint, they are clearly visible. This is unusual as prenatal enamel usually forms regularly and consistently. In this case however, these lines may be indicative of maternal ill health (see Twin A and Twin B Health Histories below). From day 18–19 after mineralisation had commenced, the first striae is visible. From this time another striae occurs approximately every week until the neonatal line is encountered.

The number of these prenatal accentuated lines differs between the twins; there are 11 visible in Twin A while only nine are visible in Twin B. Even when the acetate from Twin A was placed over the corresponding region in Twin B and the ground section was consulted again, still only nine striae were visible in the section from Twin B. Although there are two fewer of these lines expressed in the prenatal enamel of Twin B, the occurrence of all of the other accentuated prenatal striae corresponds with those in the enamel of Twin A; it is possible that they are just not visible in this section. Furthermore, there is 'potential space' in the enamel where these two lines 'would' occur. If they were visible then their position would be at the equivalent level of 34 days and 59 days as they are in Twin A.

6.2. Postnatal enamel

The results obtained for the postnatal formation times can be found in Table 8. This includes the predicted times derived from the formulae and the daily cross-striation counts. The striae in the postnatal enamel are much less regularly spaced than those in the prenatal enamel and unlike the prenatal enamel, some lines appear more pronounced than others. The postnatal formation times of the striae nearest to the enamel surface for both twins was calculated using the regression formulae and this resulted in 129 days of postnatal enamel formation for Twin A and a corresponding cross-striation count of 132 days and 126 days for Twin B with a corresponding cross-striation count of 120 days. Again the comparison of the results obtained for both twins was similar. The results obtained by the use of the regression formulae and the daily counts were also similar for both twins. For this 'final' striae for Twin A there was a discrepancy of three days between the formulae and the direct daily counts and for Twin B there was a discrepancy of six days, however this still fell within the 120–131 day 95% confidence limit range. On five occasions in Twin A and seven in Twin B the mean formulae results and the daily counts matched exactly (shown in bold in Table 8). Again on no occasion do the corresponding direct counts fall outside of the range of the 95% confidence limits.

6.3. Comparison of the photomontages with the known medical history

The results obtained using the regression formulae for the both prenatal and postnatal enamel for both twins can be found in Table 9. The results obtained using the formulae were then compared to the known medical histories for each individual. Days when a direct comparison could be made between the medical history and the striae location are highlighted in grey in Table 9.

The medical history was only revealed after the histological analysis was completed and is included below.

6.4. Twin A and Twin B health histories

Male twins were born by emergency C-section about 6pm (counted as day zero)*. At a doctor's visit earlier that day, Twin B was judged to be in distress. Twins were 32 weeks gestation.*

Twin B's growth had flat-lined since about 30 weeks as measured on ultrasound. At birth Twin B was small, only 956 g and the placenta was small. The reason was presumed to be anti-phopsholipid antibody syndrome (a clotting disorder of particular significance in pregnancy)*, for which the mother was treated throughout pregnancy with heparin and aspirin. Weekly steroid injections were also administered three weeks before birth, in order to 'pull the twins' lung development along a little faster'. Twins are both A+ the mother is O+. Twin A was a reasonable size for date, born at 1765 g.*

Feeding diaries span days 26 to about 200; after that both twins are more robust, are eating some solid food and the diaries stop.

6.5. Events shared

Day 0: *birth. Both twins were put on a ventilator over the first night but taken off sometime on Day 1. Both kept in NICU (neonatal intensive care unit) at a major university hospital. They were judged to be doing well and were quickly graduated from rooms 1–3* (graded in intensity of care).
Day 7: *Hepatitis B immunisation.*
Day 12: *Transferred by ambulance to a different hospital with a step-down special care unit because they were too well to stay in the NICU.*
Day 24/26: *Twin A home from hospital on day 24; Twin B home on day 26.*
Note: *Day 58 was their predicted due date for a 40 week gestation.*
Day 72: *DPT, Hepatitis B, H influenza type B and Polio.*
Day 72–76: *Both twins were given Paracetamol* (acetaminophen) *over five days* (notes don't say why, but suggest fever or indication of pain or discomfort following immunisations).
Day 126: *DPT, H influenza type B and Polio IPV immunisations. Both twins get Paracetamol that day; Twin B gets Paracetamol the next day also.*
Day 191: *DPT, H influenza type B immunisations. Both babies get Paracetamol that day.*

6.6. Extra events for Twin B

Day 35–40: GI upset on days 35–36; notes say Twin B is also receiving eye medicine (days 35–42) and on day 40 is very fussy.
Day 56–59: *Taken to Accident and Emergency late on day 56; Twin B has surgery for intestinal hernia on day 57; remains ill – vomiting through day 58, receiving Paracetamol through day 59.*

Table 8
Predicted mean results of the combined regression formulae when applied to the postnatal enamel of two ground sections of the deciduous first molar teeth from two individuals.[a]

Measurements from photomontage					Confidence Limits		
Measurement	Prism length (mm)	Magnification factor (mm)	Prism length (μm)	Mean (days) predicted	95% Lower (days)	95% Upper (days)	Corresponding cross-striation direct counts
Twin A							
A	8.16	2.24	18.32	8	5	10	7
B	21.26	2.24	47.62	**15**	13	18	**15**
C	29.48	2.24	66.04	**20**	17	23	**20**
D	32.81	2.24	73.49	**22**	19	25	**22**
E	36.33	2.24	81.38	**24**	21	27	**24**
F	41.64	2.24	93.27	**27**	24	30	**27**
G	66.66	2.24	149.32	41	38	44	43
H	80.35	2.24	179.98	49	46	52	52
I	85.95	2.24	192.53	52	49	56	56
J	93.26	2.24	208.90	56	53	60	60
K	97.87	2.24	219.23	59	55	63	62
L	103.75	2.24	232.40	62	59	66	66
M	111.55	2.24	249.87	67	63	71	70
N	121.31	2.24	271.73	72	68	76	74
O	142.38	2.24	318.93	84	80	89	86
P	163.06	2.24	365.25	96	92	101	98
Q	195.27	2.24	437.40	114	109	119	116
R	221.1	2.24	495.26	129	124	134	132
Twin B							
A	8.98	2.24	20.16	8	6	11	7
B	14.91	2.24	33.40	12	9	14	11
C	30.02	2.24	67.24	**20**	18	23	**20**
D	33.74	2.24	75.58	**22**	20	25	**22**
E	44.02	2.24	98.60	**28**	25	31	**28**
F	51.12	2.24	114.51	**32**	29	35	**32**
G	56.44	2.24	126.43	**35**	32	39	**35**
H	59.3	2.24	132.83	**37**	34	40	**37**
I	86.71	2.24	194.23	53	49	56	50
J	91.42	2.24	204.78	55	52	59	54
K	96.61	2.24	216.41	58	55	62	57
L	102.49	2.24	229.58	62	58	65	61
M	107.65	2.24	241.14	65	61	68	64
N	113.13	2.24	253.41	68	64	72	67
O	120.1	2.24	269.02	72	68	76	71
P	156.35	2.24	350.22	**92**	88	97	**92**
Q	162.15	2.24	363.22	96	91	100	95
R	215.02	2.24	481.64	126	120	131	120

Mean: Enamel Formation Time (days) = 0.254 × Prism Length (μm) + 3.291.
Lower 95% Confidence Limit: Enamel Formation Time (days) = 0.248 × Prism Length (μm) + 0.951.
Upper 95% Confidence Limit: Enamel Formation Time (days) = 0.260 × Prism Length (μm) + 5.631.
[a] The crown formation times are expressed in days, as well as indicating the 95% confidence limits (rounded to the nearest decimal place). The cross-striation counts for each corresponding region are also presented to allow direct comparison of the two methods. Bold mean numbers correspond exactly between the mean regression formulae counts and the direct daily counts.

DPT is a combination of vaccines which immunise against diphtheria, pertussis (whooping cough) and tetanus. The vaccine component includes diphtheria and tetanus toxoids and killed whole cells of the organism that causes pertussis.

A direct comparison between the timing of the occurrence of the accentuated striae and the corresponding events from the medical history can be found in Table 10.

7. Discussion – part 2

From the initial observation of the ground sections it was apparent that Twin B had undergone more severe postnatal stress than Twin A and on closer observation of the photomontages this did indeed turn out to be the case. This was further collaborated by consultation with the medical history.

The prediction of the twin's premature birth which was calculated using the crown formation times derived from the regression formulae for first molars (see Table 7) was correct; however, the predicted time of 62 days was inaccurate. Birth occurred after 32 weeks of an expected 40 week gestation making the twins eight weeks (56 days) premature, which results in a discrepancy of six days between the predicted time of birth and the actual time of birth.

The three separate occasions that each twin was given an immunisation/vaccination injection can be clearly identified on the photomontages (see Fig. 4). From Table 10 it can be seen that for Twin A using the time derived from the formulae for the first injection (day 7), there is a discrepancy of one day, while the cross-striation counts correspond exactly with the medical history. For the second injection (day 72), the formulae derived time corresponds exactly and the daily count is inconsistent by two days. For the third injection (day 126), there is a three day discrepancy with the formulae derived time and six days with the daily cross-striation counts. In Twin B using the time derived from the formulae for the first injection (day 7), there is a discrepancy of one day, while the cross-striation counts corresponds exactly with the medical history. For the second injection (day 72), the formulae derived time corresponds exactly and the daily count is inconsistent by one day. For the third injection (day 126), the formulae derived time corresponds exactly and

Table 9
Predicted mean results of the combined regression formulae for both prenatal (A–L) and postnatal (A–R) enamel of two ground sections of the deciduous first molar teeth from two individuals.[a]

Twin A					Twin B				
Measurements from photomontag			Confidence limits		Measurements from photomontag			Confidence limits	
Measurement	Prism Length (μm)	Mean (days) Predicted	95% Lower (days)	95% Upper (days)	Measurement	Prism Length (μm)	Mean (days) Predicted	95% Lower (days)	95% Upper (days)
A	60.95	19	16	21	A	57.59	18	15	21
B	81.00	**24**	21	27	B	79.79	**24**	21	26
C	103.82	30	27	33	C	110.19	31	28	34
D	120.78	34	31	37					
E	141.84	**39**	36	43	D	140.81	**39**	36	42
F	160.99	44	41	47	E	156.87	43	40	46
G	180.25	49	46	52	F	172.17	47	44	50
H	200.50	**54**	51	58	G	200.84	**54**	51	58
I	218.38	59	55	62					
J	235.92	**63**	59	67	H	235.49	**63**	59	67
K	255.96	68	64	72	I	260.15	69	65	73
L	293.78	**78**	74	82	J	294.36	**78**	74	82
A	18.32	**8**	5	10	A	20.16	**8**	6	11
B	47.62	15	13	18	B	33.40	12	9	14
C	66.04	**20**	17	23	C	67.24	**20**	18	23
D	73.49	**22**	19	25	D	75.58	**22**	20	25
E	81.38	24	21	27					
F	93.27	27	24	30	E	98.60	28	25	31
					F	114.51	32	29	35
					G	126.43	35	32	39
					H	132.83	37	34	40
G	149.32	41	38	44					
H	179.98	49	46	52					
I	192.53	52	49	56	I	194.23	53	49	56
J	208.90	56	53	60	J	204.78	55	52	59
K	219.23	59	55	63	K	216.41	58	55	62
L	232.40	**62**	59	66	L	229.58	**62**	58	65
					M	241.14	65	61	68
M	249.87	67	63	71	N	253.41	68	64	72
N	271.73	**72**	68	76	O	269.02	**72**	68	76
O	318.93	84	80	89	P	350.22	92	88	97
P	365.25	**96**	92	101	Q	363.22	**96**	91	100
Q	437.40	114	109	119					
R	495.26	129	124	134	R	481.64	126	120	131

[a] The crown formation times are expressed in days, as well as indicating the 95% confidence limits (rounded to the nearest decimal place). The grey highlighted numbers correspond to identifiable points in the medical histories. Bold mean numbers correspond exactly between Twin A and Twin B.

there is a discrepancy of six days with the daily cross-striation counts.

What is very apparent from this study is that there are many incremental disturbances that can be identified in the developing enamel that are not recorded in the medical notes. Some events that parents or clinical observers may think are significant may leave no markings but others they don't recognise or ignore may actually cause disruption to developing enamel. Another thing to bear in mind is that the enamel maturation process may change or disguise an event, for example, the neonatal line, which may be hypomineralised at birth, may become equally as mineralised as the surrounding enamel during enamel maturation; with only the original crystallite size and orientation remaining to identify the event in polarised light.

Despite the inevitable nature of all but the most careful of written medical histories, the results of the histological analysis of Twin A and Twin B, together with their medical histories, provide considerable support for the usefulness and accuracy of the regression equations derived from this work.

An important finding of this part of the study is that childhood inoculations often – if not always – may leave a mark in enamel. This fact is extremely useful and the basis of a carefully controlled study now exists. If comprehensive written clinical histories can be released with ethical permission, these can be used to confirm or dispute the rates of enamel formation using these 'labels' created by immunisation/vaccination at known ages. This fact may also explain similar lines that have often been noted in permanent enamel (within the cuspal enamel of first permanent molars) which may now be explained and made use of in new ways.

The estimation of the time of occurrence of 'stress lines' or 'immunisation lines' in developing enamel can be useful in the personal identification of juvenile human remains. The use of the ages of 'stressful events' could also be used to help determine the minimum number of individuals present in a skeletal assemblage if several deciduous teeth are present, for example, by matching the ages of and between these accentuated striae, two teeth that do not correspond chronologically may be indicative that they are from different individuals. Furthermore the identification of separated twins by matching corresponding accentuated incremental lines in prenatal enamel, as demonstrated by this study, may also be useful in helping to establish identity.

8. Conclusions

This study presents new data for pre- and postnatal deciduous enamel formation times for each tooth type. The regression formulae used to establish these times and the 95% confidence limits were derived from four teeth of each tooth type. Cumulative cross-striation counts were recorded from six prism path

Table 10
Predicted mean results of the combined regression formulae for the postnatal enamel of two ground sections of the deciduous first molar teeth from two individuals.[a]

Twin A							Twin B						
Measurement	Mean (days) predicted	Confidence limits		Corresponding cross-striation direct counts	Corresponding medical history	Day	Measurement	Mean (days) predicted	Confidence limits		Corresponding cross-striation direct counts	Corresponding medical history	Day
		95% Lower (days)	95% Upper (days)						95% Lower (days)	95% Upper (days)			
					Birth placed on ventilator	0						Birth placed on ventilator	0
					Removed from ventilator	1						Removed from ventilator	1
A	8	5	10	**7**	Hepatitis B immunisation	**7**	A	8	6	11	**7**	Hepatitis B immunisation	**7**
B	15	13	18	15	Transfer from NICU to step-down unit	12	B	**12**	9	14	11	Transfer from NICU to step-down unit	**12**
C	20	17	23	20			C	20	18	23	20		
D	22	19	25	22			D	22	20	25	22	Discharged from hospital	26
E	**24**	21	27	**24**	Discharged from hospital	**24**							
F	**27**	24	30	**27**			E	28	25	31	28		
							F	32	29	35	32		
							G	**35**	32	39	35	Gastro-intestinal problems	**35**–36
							H	37	34	40	37	Eye problems	35–42
G	41	38	44	43									
H	49	46	52	52									
I	52	49	56	56			I	53	49	56	50		
J	56	53	60	60			J	55	52	59	54	Admitted to A and E	56–59
												Surgery for hernia	57
K	59	55	63	62			K	**58**	55	62	57	Vomiting	**58**
L	62	59	66	66			L	62	58	65	61	Received Paracetamol	59
							M	65	61	68	64		
M	67	63	71	70			N	68	64	72	67		
N	**72**	68	76	74	DPT, Hepatitis B, H influenza type B, Polio	**72**	O	**72**	68	76	71	DPT, Hepatitis B, H influenza type B, Polio	**72**
					Received Paracetamol	72–76						Received Paracetamol	72–76
O	84	80	89	86			P	92	88	97	92		
P	96	92	101	98			Q	96	91	100	95		
Q	114	109	119	116									
R	129	124	134	132	DPT, H influenza type B, Polio and Paracetamol	126	R	**126**	120	131	120	DPT, H influenza type B, Polio and Paracetamol	**126**

[a] The crown formation times are expressed in days, as well as indicating the 95% confidence limits (rounded to the nearest decimal place). The cross-striation counts for each corresponding region are also presented to allow direct comparison of the two methods. The grey highlighted numbers correspond to points in the medical histories and the bold mean regression formulae counts and cross-striation counts correspond exactly with events in the medical histories.

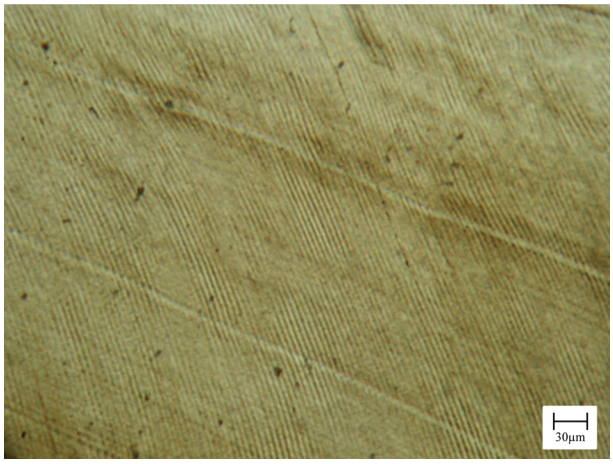

Fig. 4. This figure shows an example of two accentuated 'vaccination lines' in the enamel of a deciduous first molar from an individual with a known medical history, in this case these accentuated lines were caused by DPT vaccinations.

trajectories in 20 ground sections ($n = 120$). The resultant crown formation times derived by the use of these formulae corresponded well with previously published estimates.[10,12,14,29]

The use of these regression formulae to calculate pre- and postnatal as well as total enamel formation times, was designed to allow the calculation of enamel formation times without having to count daily cross-striations, therefore saving forensic osteologists and anthropologists time when estimating the age of unidentified juvenile remains, in order to help procure a positive identification.[3,5] This is particularly important in forensic work when the police require a speedy response as to the age of an individual. A limitation of the use of these regression formulae to estimate the age at death of juvenile remains is that they can only be applied to teeth where the crown is still in the process of forming. This technique is therefore limited to the maximum age at which deciduous second molar enamel is still forming (~55 weeks postnatally), however, age estimates of individuals older than this can be made using permanent tooth crowns.[6]

A test of the usefulness of these formulae on two teeth belonging to individuals with known medical histories of stress events during their early lives provided good correspondence between estimated times of stress events and the known chronological ages of the events. It was not possible however, to match the nature of the stress factor with the kind of marking in the enamel of each tooth studied and some additional accentuated markings suggest that not all stress events were either noticed, or considered significant enough for records to be kept of them.

Being able to estimate the time of occurrence of 'stress lines' or 'immunisation lines' in developing enamel can be useful in the personal identification of juvenile human remains. The use of the formulae developed here to identify such occurrences, resulted in a maximum difference of only three days (95% confidence limit range of ten days) between the estimated day of a stress event (immunisation) and the record of the event in the medical history. For this same incident there was a difference of six days between the direct cross-striation count and the record of the event. If comparative medical histories are available then this could be invaluable in the confirmation or elimination of a possible identification. Likewise, the occurrence of premature birth, also identified using these formulae, could assist in the identification of juvenile remains if a comparative medical history is available.

These regression formuale make it possible to estimate prenatal, postnatal and total enamel formation times from either forensic or archaeological deciduous ground sections that do not exhibit clear daily cross-striations. Daily cross-striations are often poorly preserved or invisible in deciduous enamel and clear incremental markings are often hard to find. Furthermore, the process is quite time consuming, however the approach outlined in this study makes such estimates possible by utilizing regression formulae, the prerequisites being that the direction of the enamel prism path is visible and there are sufficient accentuated markings visible in the lateral enamel in order to track enamel formation from the region of the dentine horn to the cervix.

The methods proposed here offer a solution to the aging (and thereby possible identification) of unknown human juvenile remains. They also have the potential to form the basis of further carefully controlled studies to calculate rates of enamel formation using the 'labels' created by stress/vaccinations/immunisations when these occur at known ages. Combined with written medical histories, obtained with ethical permission, the methods and approaches described in this study offer a new and powerful way of studying deciduous tooth formation in the future.

Conflict of interest

None declared.

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None.

Ethical approval

None.

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